FINAL SYNOPSIS OF Ph.D. THESIS

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Biochemical response of coconut (*Cocos nucifera* L.) seedlings to elevated [CO_2] and temperature

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1. Introduction

Climate change refers to a change of climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere due to increased green house gases (IPCC 2007a). The main characteristics of climate change are increases in average global temperature. As a result of global warming, increase in the frequency and intensity of heat waves and heavy precipitation events, drought and floods are projected. All these changes in climate have profound influence on agriculture. Studies indicate that productivity of most of crops in tropical region is going to decline due to climate change. Reports of the IPCC and other global studies indicate a probability of 10-40 % loss of crop production in India with increases in temperature 2080-2100 (Parry *et al*., 2004; IPCC 2007 b).

Coconut, being perennial in nature will have to face the changes in climatic conditions even in a single life span of 60 years. Like any crop plant, coconut also responds to the external factors and climate change is increasingly considered to be the most important external factor which influences the productivity. Variation in the range of temperature and increased concentration of [CO_2] are the two major climatic factors that can affect the productivity of coconuts (Naresh Kumar, 2008). Simulation analysis indicated that climate change is likely to increase coconut yields in western coast of India while affecting the yields in eastern coastal areas (Naresh Kumar *et al*., 2008a; Naresh Kumar, 2008; Naresh Kumar and Aggarwal, 2009).

It is well established that all plants have evolved many adaptation to grow, develop and reproduce in a narrow range of environmental conditions. The major climatic factors include [CO_2] and temperature, apart from rainfall, which influence the plant growth and development. The response of plant metabolism to these factors can be assessed by monitoring the biochemical parameters. Information on biochemical response of plants to

external factors not only provides insights on the ability of the species to adapt to altered environment but also gives the clues for interventions for possible crop improvement. Further, the biochemical studies also provide clues for possible changes in leaf defense capability against pests and diseases. Even though information on the biochemical response of plants under elevated [CO_2] and temperature condition is known for some annuals, there is no such information available for coconut palms. Understanding the biochemical mechanisms that make some varieties tolerant compared to others is fundamental in identifying clearly recognizable traits for use in breeding programs aimed at developing cultivars adapted to the environmental stresses (Laila and Abel, 2002).

Hence the present study is conducted with the objectives:

1. To study the leaf biochemical composition of coconut seedlings under elevated [CO_2] and temperature.
2. To study the scavenging enzyme activity in leaves of coconut seedlings
3. Qualitative and quantitative analysis of leaf total soluble protein and thermo-stable proteins in coconut seedlings.
4. To study the soil enzyme activity and carbon nitrogen metabolism in rhizosphere soil under elevated [CO_2] and temperature.

2. Brief review of literature

The experiments conducted in controlled environment facilities such as Free Atmospheric [CO_2] Enrichment (FACE) or Open Top Chambers (OTC) indicated that elevated [CO_2] increase the concentration of leaf carbohydrates while higher temperatures decrease it (Sasmita *et al.,* 2008). Elevated [CO_2] and temperature increase amino acid in leaves (Ainsworth *et al*., 2007). Increases in the atmospheric [CO_2] causes the changes in carbon based secondary compounds like phenolics (Bryant *et al.,* 1983). Increase in concentration of phenolic compounds under elevated [CO_2] and temperature conditions has been reported (Penuelas *et al.,* 1996). Plants grown in elevated [CO_2] had high concentrations of starch while increase in temperature caused a decrease of it (Chen *et al.,* 2004). Enhanced carbon dioxide increased reducing sugars (Urbonaviciute *et al*, 2006) and affected development of epicuticular wax (Vanhatalo *et al.,* 2001). Exposures

to elevated [CO_2] and elevated temperature decreased soluble proteins (Tegelberg *et al.,* 2008). Elevated [CO_2] showed no effect on production of heat-shock proteins (Sasmita *et al.,* 2008) while elevated temperature increased accumulation of heat shock proteins (Chaitanya *et al*., 2001). Studies indicated decrease in leaf catalase and super oxide dismutase activity under elevated [CO_2] (Evelyn and Neil, 1988). High temperature reduced super oxide dismutase activity at ambient [CO_2] but at the same time elevated temperature enhanced catalase activity in high [CO_2] treatments (Sasmita *et al.,* 2008). Recent report indicates increase in the activity of antioxidant enzymes in grape vines under elevated [CO_2] and temperature regimes thereby avoiding the oxidative damage in moderate drought conditions (Parra *et al*., 2011). Elevated [CO_2] reduced leaf nitrogen (Ray *et al.,* 2000) while elevated temperature increased nitrogen content (Kuokkanen *et al.,* 2001).

Not only the plant, but soil parameters also are influenced by increase in the atmospheric concentration of [CO_2] and rise in temperature. It was suggested that elevated [CO_2] can enhance enzyme activities (Ross *et al*., 1995) and stimulation of microbial extracellular enzyme activities by elevated [CO_2] depends on soil aggregate size (Dorodnikov et al., 2009) while increase in soil temperature can affect the soil micro organisms and that can affect soil enzyme activity (Scharpenseel *et al.,* 1990). Elevated [CO_2] and high temperature interaction accelerated the general turnover of the organic C fractions of the tropical rice soil and through increase in microbially mediated processes like enzymes (Das *et al*., 2011).

3. Materials and methods

Seedlings of three coconut cultivars viz., WCT, LCT, COD and two hybrids viz., WCT x COD and COD x WCT were grown in six 4m^3 Open Top Chambers (OTCs) at Central Plantation Crops Research Institute, Kasaragod (12˚18′ N, 75˚ E, ~7m AMSL). In OTCs, they were exposed separately to i) elevated [CO_2] at 550 µmol mol^{-1} in one OTC ii) elevated [CO_2] at 700 µmol mol^{-1} in another OTC and iii) elevated temperature [T] at 2^oC above control OTC in the third OTC. One set of seedlings were maintained in the fourth OTC without any additional treatment to serve as the chamber control (control OTC). Apart from these, one more set of seedlings were also maintained under shade net to

serve as the absolute control. The [CO_2] levels were maintained at set level of 550 and 700 µmol mol^{-1} with constant supply of commercial [CO_2] using the automated [CO_2] supply system and the levels were monitored by the SCADA system. Rise of temperature by 2˚C above that of control chamber was realized by using hot air blowers, which are controlled by the SCADA system. The seedlings were maintained under elevated [CO_2] and temperature conditions for three years and periodical observations were made for quantifying their biochemical responses.

3.1. Seedling biochemical parameters

Seedling biochemical parameters viz., total soluble sugar (Dubois *et al*., 1956), total free amino acids (Moor and Stain, 1948), total phenol (Bray and Thorpe, 1954), starch (Hedge and Hofreiter, 1962), reducing sugars (Somogyi, 1952), proline (Bates *et al.,* 1973) epicutiular wax (Ebercon *et al.,* 1977; Rajagopal *et al.,* 1989), epicutiular wax fatty acid profile (Padua-Resurreccion and Benzon, 1979; Naresh Kumar, 2007; GC2010, Shimadzu, Japan), protein (Bradford, 1979; Naresh Kumar *et al.,* 2007), heat stable protein fraction (HSPF) (Bradford, 1979; Naresh Kumar *et al.,* 2007), super oxide dismutase (SOD) (Beauchamp and Fridovich, 1971), peroxidase (Kar and Mishra, 1976), polyphenol oxidase (Kar and Mishra, 1976) and catalase (Kar and Mishra, 1976) were studied following the methods provided in source as indicated in parenthesis of each parameter.. Seedling total nitrogen was analyzed using nitrogen analyzer (Gerhardt vapodest, UK) (Kjeldal method 1883). Qualitative analysis of coconut total protein and HSPF using SDS PAGE was done by following method standardized for coconut tissue (Naresh Kumar *et al*., 2007).

3.2. Soil biochemical parameters

Soil biochemical parameters viz., pH of soil (Sartorius pp 20, India, Jackson, 1967), electric conductivity (Sartorius pp 20, India, Richards, 1954); dehydrogenase (Klein *et al.,* 1971); phosphatase (Tabatabai and Bremner, 1969); urease (Tabathabai and Bremner, 1972); soil amylase, invertase and cellulase (Kelly and Rodriguez, 1975); total soluble sugar (Dubois *et al.,* 1956), total phenol (Bray and Thorpe, 1954) and total free amino acids (Moor and Stain, 1948) were estimated. Soil nitrogen was analyzed by using

nitrogen analyzer Gerhardt vapodest, UK. (Kjeldal method 1883), Soil respiration was taken with LCA 4 soil respiration chamber fitted to infrared analyzer (ADC Bioscientific Ltd. UK). Soil nutrients like Iron (Fe), Copper (Cu), Zink (Zn) manganese (Mn) Calcium (Ca) and magnesium (Mg) were analyzed using atomic absorption spectroscopy (GBC Avanta ver 1.33 Australia) (Jackson, 1967; Lindsay and Norwell, 1978; Muralidharan, 1991). Potassium (K) and sodium (Na) was determined using flame photometer (Jenway PFP7, England) (Jackson, 1967; Khan *et al.,* 1982). Soil phosphorous was determined by following the modified titration method of Chang and Jackson (1967) by Biddapa and Sarkunan (1982) for rhizosphere soil of coconut.

All experimental data were analyzed statistically using SPSS software (Version 10.0.1).

4. Salient findings

Growth of coconut seedlings in OTCs was comparatively less than those grown in shade net. In order to discount the chamber effects, the biochemical response of seedlings exposed to elevated [CO_2] and temperature was compared to that of seedlings grown in control chamber. Similarly, biochemical characteristics of rhizosphere soil were compared to those of chamber control. Results indicated that elevated [CO_2] and temperature induced changes in biochemical composition of leaf tissue and the magnitude of change in concentration of various biochemical compounds progressively varied with period of exposure. There existed a significant variation among the cultivars and hybrids.

Elevated [CO_2] increased leaf total soluble sugars (26.4 % and 39.4 % at 550 and 700 µmol mol^{-1}, respectively), reducing sugar, free amino acids (28.5 % and 113 % at 550 and 700 µmol mol^{-1}, respectively), starch, proline, Root C/N ratio, specific activities of superoxide dismutase (17.6 % and 11.5 % at 550 and 700 µmol mol^{-1}, respectively), peroxidase and catalase. Similarly, activities of rhizosphere soil amylase, cellulase, soil electric conductivity, soil nutrients such as sodium (Na), potassium (K) and magnesium (Mg) increased under elevated [CO_2] treatments.

On the other hand, elevated [CO_2] reduced the concentration of total phenols (2.5 % and 2% at 550 and 700 μmol mol^{-1}, respectively), specific activity of polyphenol oxidase (~75 %), and shoot CN ratio. Activities of soil enzymes such as phopatase, dehydrogenase and urease also decreased. Under elevated [CO_2] conditions, soil pH decreased and concentration of soil nutrients such as copper (Cu), calcium (Ca), iron (Fe), zinc (Zn), phosphorus (P), manganese (Mn) and nitrogen (N) also decreased, apart from decrease in soil total sugars. Response of some parameters was not linear with concentration of [CO_2]. For example, concentration of soluble proteins and heat stable protein fraction (HSPF) increased at 500 μmol mol^{-1} [CO_2] while they deceased at 700 μmol mol^{-1} [CO_2]. Similar trends were observed for soil free amino acids, phenols, invertase activity and respiration.

Elevated temperature significantly increased concentration of leaf total free amino acids (7.1%), proline (5.4 %), HSPF (36.5 %), shoot CN ratio (29.5 %) and root CN ratio (58 %) and activities of superoxide dismutase (67.5 %) and catalase. Similarly increase was observed in activity of soil invertase, soil urease and soil cellulase and electric conductivity. On the other hand, elevated temperature significantly reduced concentration of leaf total soluble sugars (30.7 %), reducing sugars (43.8 %), total phenols (28.4 %), starch (8.4 %) and reduced activities of poly phenol oxidase (62.8 %) and that of peroxidase (36.3 %). Apart from these, soil respiration, concentration of soil biochemicals like phenols and soluble sugars and activities of soil enzymes such as amylase, phosphatase and that of dehrydrogenase reduced in elevated temperature conditions. Further, soil nutrients viz., nitrogen (N), phosphorus (P), potassium (K), sodium (Na), copper (Cu), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn) were also less in elevated temperature conditions.

The magnitude and direction of biochemical response of seedlings varied depending on cultivar and with duration of exposure. During first year, decrease in total soluble sugars concentration was observed in COD seedling in elevated temperature and [CO_2] 550 μmol mol^{-1}, respectively. Same time in [CO_2] 700 μmol mol^{-1} condition, CODxWCT showed decrease in total soluble sugar concentration. During second year, decrease in

total soluble sugar was observed in CODxWCT, LCT and WCT seedlings. Concentration of free amino acids initially increased (by the end of the first year) but prolonged exposure (by the end of second year) to elevated [CO_2] reduced their concentration as observed in COD x WCT. On the other hand, in WCT and WCTxCOD seedlings their concentration increased in elevated temperature and [CO_2] treatments. Concentration of total phenols increased in WCT seedlings under elevated [CO_2] conditions. Elevated temperature and [CO_2] conditions increased poly phenol oxidase activity in WCT while decreased in COD x WCT seedlings. The concentration of proline increased in CODxWCT and WCT seedlings under elevated temperature and [CO_2] conditions while decreased in WCTxCOD and COD. Increase in epicuticular wax (ECW) concentration was observed in WCT, LCT and COD seedlings under temperature, [CO_2] 550 µmol mol^{-1} and 700 µmol mol^{-1} conditions, respectively. On the other hand, ECW concentration decreased in CODxWCT and WCTxCOD.

Activities of scavenging enzymes were also influenced by elevated [CO_2] and temperature conditions and response varied depending on cultivar as well. For instance, activity of peroxidase increased in WCT, LCT and COD seedlings under elevated temperature, [CO_2] 550 µmol mol^{-1} and [CO_2] 700 µmol mol^{-1} treatments, respectively, while it reduced in WCTxCOD seedlings in both conditions. The catalase activity increased in CODxWCT and LCT seedlings under elevated temperature and [CO_2] treatments while it decreased in WCT and COD seedlings. Activity of superoxide dismutase increased in LCT and CODxWCT seedlings in elevated temperature and [CO_2] conditions, while it decreased in WCT seedlings.

The elevated temperature and [CO_2] conditions increased concentration of heat stable protein fraction (HSPF) in COD and CODxWCT seedlings while they decreased in LCT and WCTxCOD. Concentration of proteins increased in COD while decreased in COD x WCT (due to elevated temperature) and in LCT (in elevated [CO_2]). On the other hand, root CN ratio increased in WCTxCOD (in elevated temperature and [CO_2]) and decreased in COD (in elevated temperatures) and WCT (in elevated CO_2) seedlings. However, increase in shoot CN ratio was observed in COD, WCTxCOD and LCT seedlings in

elevated temperature, [CO_2] 550 µmol mol^{-1} and [CO_2] 700 µmol mol^{-1} treatments, respectively; while it decreased in WCT seedlings.

5. Brief discussion

Seedlings exposed elevated [CO_2] had higher concentration of reducing sugar, soluble sugars and starch indicating higher photosynthetic fixation of [CO_2] to stable energy-rich compounds such as glucose and sucrose and starch. Availability of additional carbon skeletons seems to have stimulated the synthesis of amino acids as indicated by increased concentration of free amino acids and proline. This may be due to the increased photosynthesis, non-enzymatic detoxification of free radicals and nitrogen uptake in elevated [CO_2] conditions (Alia *et al.,* 2001; Srivastava *et al.,* 2002; Schimel and Bennett, 2004; Ainsworth *et al*., 2007). Increased accumulation and amino acids also helped to synthesize more proteins as indicated by total soluble protein concentration under elevated [CO_2] treatment and increased HSPF in total soluble protein under temperature treatment indicate the adaptive capacity of coconut seedlings to elevated [CO_2] and temperature conditions. At the same time phenol concentration decreased in elevated [CO_2] and [T]+2^oC condition as changes in the atmospheric [CO_2] and temperature cause changes in carbon based secondary compounds (CBSC) like phenolics (Bryant *et al*., 1983; Akin *et al.,* 1987; Ray *et al*., 2003). If concentration of phenolic compounds reduced below a threshold can predispose coconut seedlings to pest attack. Elevated [CO_2] and temperature affected the development of the epicuticular waxes in coconut seedlings as in other crops (Paoletti *et al.,* 1998; Vanhatalo *et al*., 2001).

Elevated temperature and [CO_2] conditions not only directly influence the seedling biochemical composition but also influence the soil biochemical properties, which in turn influence the seedlings growth, development and also biochemical composition. Activities of soil enzymes and amount of available soil nutrients have strong correlation with population and growth of soil microbes and also on the amount of organic substrates entering soil. Increase in [CO_2] influenced activities of soil enzymes an such as cellulase, phosphatase, amylase, invertase, urease and dehydrogenase may be due to decreased soil

pH in elevated [CO_2] conditions as dissolving of [CO_2] in water forms carbonic acid. At the same time, soil EC increased which was inversely proportional to decrease in soil pH. When [CO_2] dissolves in water it forms not only carbonic acid (H_2CO_3) but also it forms bicarbonate ions (HCO_3-) and carbonate ions (CO_3-) this caused increase in soil EC. In elevated temperature treatment, there was no such change observed as compared to that in rizosphere soil in chamber control and in shade net. Influence of elevated [CO_2] on activities of soil cellulase (Jennifer *et al.,* 2002), phosphatase (Paul and Clark, 1996), amylase (Stitt and Krapp, 1999), invertase (Jia *et al.,* 2005), urease (Templer and Dawson, 2004; Joachim *et al.,* 2008) and dehydrogenase (Jia *et al.,* 2005) were reported. Variations in the nutrient uptake and soil nutrient concentration was observed due to the variations in seedling requirement, availability of nutrients to soil and altered soil enzyme activity due to increased [CO_2] and temperature (Cakmak and Engel, 2002).

6. Summary and conclusions

The coconut seedlings, when grown in elevated [CO_2] and temperature conditions, have shown biochemical responses resulting in variation in concentrations of total soluble sugar, reducing sugar, starch, protein, HSPF, epicuticular wax and fatty acid profile of ECW. Increase in the fraction of HSPF in total proteins in elevated temperature conditions indicates the adaptive capacity of coconut to projected increase in temperatures in future climate scenarios. Results indicated that elevated [CO_2] and temperature reduced total phenolic concentration in majority of cultivars, which may pre-dispose coconut to pests and diseases in future scenario. Overall results indicate that in climate change scenarios, WCT is likely to remain relatively more tolerant while COD, LCT and COD x WCT may become more predisposed to pests and diseases. In climate change scenarios, where rise in temperature is associated with the increased levels of [CO_2] in atmosphere, accumulation of high levels of proline as a result of higher [CO_2] conditions may prove beneficial for coconut for tolerating the high temperature stress. Changes observed in soil nutrient concentration indicate the influence of [CO_2] and temperature on absorption pattern. The soil nitrogen uptake increased in the rhizosphere soil of coconut due to the elevation of [CO_2] indicating higher requirement of fertilizer application in future scenarios. The accumulation of foliar free amino acids also is an

adaptive measure of coconut in elevated [CO_2], to detoxify the accumulated ammonia due to increased up take of nitrogen or serve as an additional nitrogen source. Changes in the activity of soil enzymes and soil respiration indicate alteration in soil microbial population. Results indicate that coconut seedlings have capacity to adapt to future climate through changes in biochemical mechanisms that make some varieties tolerant compared to others.

7. Publications

John Sunoj, V. S., Naresh Kumar, S. and Muralikrishna, K. S. 2009. Proline may assume greater role in coconut adaptation to elevated CO_2 and temperature conditions. Proceedings of national workshop on climate and development at Kerala Agricultural University, Thiruvananthapuram, PP: 159-166.

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